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Energy expenditure at rest and during sleep in children with Prader-Willi syndrome is explained by body composition^{1,2}

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ABSTRACT

Background: Obesity in Prader-Willi syndrome (PWS) seems to be related to a low basal metabolic rate (BMR). In addition, abnormal sleep patterns reported in PWS might affect sleeping metabolic rate (SMR).

Objective: Our objective was to assess BMR and SMR adjusted for fat-free mass in young PWS patients.

Design: Subjects were 17 PWS patients (10 females and 7 males aged 7.5–19.8 y) and 17 obese control subjects matched for sex and bone age. SMR was measured in a respiratory chamber, BMR with a ventilated-hood system, and body composition by deuterium dilution.

Results: BMR and SMR were significantly lower in the PWS group than in the control group (5.36 ± 1.18 and 4.62 ± 1.08 MJ/d compared with 6.38 ± 1.55 and 5.60 ± 1.52 MJ/d, respectively; $P < 0.05$). When fat-free mass was included in the analysis, multiple regression showed no differences in BMR and SMR between groups. When weight was included in the analysis instead of fat-free mass, SMR was lower in the PWS group. Fat-free mass was lower in the PWS group both as an absolute value and when adjusted for height.

Conclusion: BMR and SMR are low in young patients with PWS because of a low fat-free mass. *Am J Clin Nutr* 2000; 71:752–6.

KEY WORDS Prader-Willi syndrome, obesity, child, energy metabolism, body composition, bone age, fat-free mass, basal metabolic rate, sleeping metabolic rate

INTRODUCTION

Prader-Willi syndrome (PWS; 1) is a clinically diagnosed genetic disorder that results from the absence of normally active paternally inherited genes at chromosome 15(q11–q13) (2). About 70–75% of the patients have an interstitial deletion in this region. The majority of the remaining PWS patients have a maternal uniparental disomy for chromosome 15. The major clinical criteria (3) for the diagnosis of PWS include neonatal and infantile central hypotonia with feeding problems in infancy, hypogonadism, and global developmental delay resulting in mild-to-moderate mental retardation (4). Other symptoms, such as short stature, altered temperature sensitivity, high pain threshold, and characteristic behavior problems (eg, temper tantrums

or obsessive-compulsive behavior), support the hypothesis that dysfunction of various hypothalamic systems are the probable pathophysiologic basis of PWS (5–7). PWS is also known as the most common human genetic disorder linked to obesity (8). The high prevalence of obesity in PWS is likely caused by a combination of a low energy expenditure and an almost insatiable hunger that starts in early childhood (9, 10).

Schoeller et al (11) were the first to report that common formulas that use age, sex, height, and weight to predict basal metabolic rate (BMR) would overestimate BMR in adult PWS patients. They found that the Cunningham formula (12), which uses fat-free mass (FFM) in the equation, did not significantly overestimate BMR. Later studies, however, either could not confirm the reduced BMR in young PWS patients (13) or found a reduced BMR, even when BMR was adjusted for FFM (14). The question, therefore, of whether BMR adjusted for FFM is reduced in young PWS patients is still unanswered.

BMR is composed of 2 components, sleeping metabolic rate (SMR) and arousal. The respiratory chamber measures SMR overnight and offers an alternative measurement of metabolic rate in the inactive and fasted state. In addition, parents and caregivers often report excessive sleepiness, daytime hypersomnolence, and sleep disturbance at night in PWS patients. Studies have shown abnormal sleep and sleep cycles with abnormal rapid eye movement (REM) and multiple brief REM periods, suggesting a specific disruption of the timing of REM–non-REM cycles (15–17), possibly causing an alteration in SMR in patients with PWS.

As a result of contradictory reports of BMR and of abnormal sleep patterns in patients with PWS, the aim of our study was to measure BMR and SMR adjusted for FFM in children and adolescents with PWS in comparison with those of obese control subjects.

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TABLE 1

Body composition, energy expenditure, and other characteristics of subjects with Prader-Willi syndrome (PWS) and control subjects¹

	PWS (n = 10 F, 7 M)	Control (n = 10 F, 7 M)
Bone age (y)	12.7 ± 2.9	12.7 ± 3.2
Age (y)	11.9 ± 3.4	11.3 ± 2.6
Height (m)	1.43 ± 0.16	1.49 ± 0.20
Weight (kg)	50.0 ± 19.7	61.5 ± 25.6
FFM (kg)	27.5 ± 9.9 ²	35.9 ± 13.4
FM (kg)	22.4 ± 11.7	25.6 ± 12.7
BMI (kg/m ²)	23.5 ± 6.0	26.0 ± 6.5
FFMI (kg/m ²)	12.9 ± 2.3 ³	15.4 ± 2.7
FMI (kg/m ²)	10.6 ± 4.5	10.6 ± 4.0
SMR (MJ/d)	4.62 ± 1.08 ²	5.60 ± 1.52
BMR (MJ/d)	5.36 ± 1.18 ²	6.38 ± 1.55

¹ $\bar{x} \pm \text{SD}$. FFM, fat-free mass; FM, fat mass; FFMI, fat-free mass index; FMI, fat mass index; SMR, sleeping metabolic rate; BMR, basal metabolic rate.

^{2,3}Significantly different from control group (independent-samples *t* test): ²*P* < 0.05, ³*P* < 0.01.

SUBJECTS AND METHODS

Subjects

Seventeen PWS patients (aged 7.5–19.8 y) were recruited with the assistance of the Dutch Prader-Willi Association. The subjects were assessed according to the Holm criteria (3). The Holm system provides a quantitative measure of PWS symptoms. PWS was preferably confirmed by either a deletion on chromosome 15 or uniparental disomy. When only clinical data were available, a critical evaluation was done by the same clinical geneticist. The PWS subjects were sex- and bone age-matched with healthy obese control subjects (aged 6.3–15.3 y) recruited from the regional public health department. Bone age was determined by assessing epiphyseal maturation by the same pediatric endocrinologist using an X-ray of the midportion of the left hand and standard growth data (18). None of the PWS patients was receiving hormone therapy or treatment with human growth hormone before or during the study. Control subjects with endocrine causes or other secondary causes of obesity were excluded. Subject characteristics are shown in **Table 1**.

Before the start of the study the parents gave written, informed consent confirmed by oral approval of the child. The study was approved by the Medical Ethical Committee of Maastricht University.

Methods

SMR was measured overnight in a respiratory chamber. After this, BMR and body composition were determined by ventilated hood and deuterium dilution, respectively. The subject and parent were invited to come to the laboratory at 1900 after a normal dinner. At 2200, the subject entered the respiratory chamber. The hours in between were used to familiarize the subject with the study equipment, do a practice session with the ventilated hood, and produce a background urine sample for body-composition analysis.

Body composition was measured with a dilution technique according to the Maastricht protocol (19). In summary, the subject received an orally administered dose of 0.1 g D₂O/L total

body water (TBW), diluted in 75 mL tap water, as the last drink before bedtime. TBW was estimated from age- and sex-specific formulas (20). After dosing, the subject went into the respiratory chamber and was asked to go to sleep before 2230. A urine sample was taken from the second voiding the next morning, 10 h after dose administration. Isotope abundance in urine was determined with an isotope ratio mass spectrometer.

TBW was calculated as the ²H dilution space divided by 1.04 to correct for exchange of the ²H label with nonaqueous hydrogen of body solids (21). FFM was assessed with the assumption that FFM contained all body water. Hydration factors of FFM were based on maturation-specific values (22). Maturation was assessed according to Tanner's puberty ratings (23). Fat mass (FM) was calculated by subtracting FFM from the subject's total body weight. To allow for comparisons between subjects, FM and FFM were expressed as indexes: FFMI (kg/m²) and FMI (kg/m²), respectively. Patients with PWS experience specific periods of growth stunting, leading to changes in age-related body composition. In this way, we corrected for the large variation in height, in analogy with body mass index (BMI; in kg/m²): BMI = FFMI + FMI.

SMR was measured during a 12-h overnight stay in the respiratory chamber, which was described in detail previously (24). The respiratory chamber, an open-circuit indirect calorimeter (14 m³), was maintained at a constant air temperature of 18°C during the night. The chamber was ventilated with fresh air at ≈40 L/min. Activity of the subject was measured by using an analogue ultrasound radar system. SMR was calculated automatically according to de Weir (25), between 2300 and 0630, over the 3-h interval with the lowest radar count. The next morning, the subject came out of the respiratory chamber at 0630 to void and immediately returned to bed for BMR measurement in an adjacent room. Because the subject was not active that morning, the BMR measurement was started after they laid supine for 10 min.

Oxygen consumption and carbon dioxide production were measured by a computerized, open-circuit, ventilated-hood system between 0700 and 0800 for 40–50 min, while the subject was lying supine watching television. Gas analyses were performed by using a paramagnetic oxygen analyzer (Servomex, Crowborough, United Kingdom) and an infrared carbon dioxide analyzer (Servomex). BMR was calculated according to de Weir's formula over the 14-min interval with the lowest SD.

The body weight of subjects was measured on an electronic scale (model E1200; Mettler, Greifensee, Switzerland) before the subjects consumed any food or drink, after voiding, and while wearing underclothing. The height of subjects was measured by using a stadiometer.

Statistical analysis

Differences between the independent variables of the PWS group and those of the control group were analyzed by using the two-sample *t* test. The grouping variable PWS was defined as PWS patients = 1, control subjects = 0. A multiple linear regression model with BMR as the dependent variable and bone age, FFM, FM, sex, and PWS status as independent variables was used to analyze the differences between groups adjusted for these independent variables. First, the difference in regression slope of the influence of FFM on BMR was tested by using an interaction variable of PWS and FFM (PWS × FFM) after correction for the variables in the equation. Second, the difference between groups, again corrected for these independent variables, was estimated and tested for significance by using linear regression, assuming

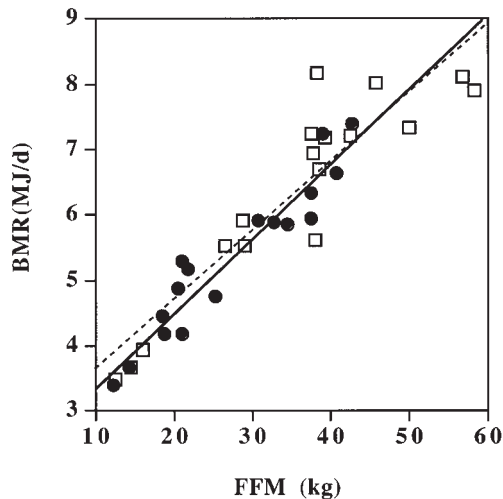


FIGURE 1. Basal metabolic rate (BMR) as a function of fat-free mass (FFM) plotted for the Prader-Willi syndrome (PWS; ●) and control (□) groups. The regression equation for PWS patients: $\text{BMR} = 0.11 \text{ FFM} + 2.21$ ($R^2 = 0.91$); for control subjects: $\text{BMR} = 0.11 \text{ FFM} + 2.60$ ($R^2 = 0.83$).

equal slopes. This analysis was also done for SMR as the dependent variable, but now with weight, bone age, sex, and PWS status as the independent variables by using an interaction variable of PWS status and weight ($\text{PWS} \times \text{weight}$). The significance level was chosen at 5%. Data were expressed as means \pm SDs. SPSS, release 6.1, for Macintosh (SPSS Inc, Chicago) was used.

RESULTS

Clinical characteristics of PWS patients and control subjects are shown in Table 1. There were no significant differences in age, height, weight, or BMI between groups. FFM and FFMI were significantly lower in the PWS group, and FM and FMI were similar in both groups. SMR and BMR were also significantly lower in the PWS group than in the control group. FFM was plotted against BMR in Figure 1. When BMR was expressed as a function of FFM in separate linear regression lines for the PWS and control groups, the R^2 values were 0.91 and 0.83, respectively. These lines were not significantly different, as was evident from multiple regression when BMR was predicted by bone age, FFM, FM, and sex with PWS status as the grouping variable. The coefficients of bone age and FM were also not significant (Table 2). When SMR was plotted as a function of FFM, the regression lines of the PWS group and the control group were remarkably similar (Figure 2). In Table 3 the observed variability in SMR was explained by the variables bone age, sex, weight, and PWS status, resulting in a significant difference in SMR between groups. FFMI was plotted as a function of FMI (Figure 3). In this plot, the difference between the regression lines was significant. Because the interaction variables $\text{PWS} \times \text{FFM}$ and $\text{PWS} \times \text{weight}$ were not significant in the regression analyses, these variables were not included in the related tables.

DISCUSSION

The present study showed that children and adolescents with PWS had a lower energy expenditure both during rest and during

TABLE 2

Results of multiple linear regression analysis of the influence of bone age, fat-free mass, fat mass, sex, and Prader-Willi syndrome (PWS) on basal metabolic rate (BMR)

Variable	β Coefficient ¹	SE	95% CI β ²	P
Bone age (y)	0.017	0.055	(-0.095, 0.129)	0.756
FFM (kg)	0.096	0.017	(0.061, 0.131)	0.000
Fat mass (kg)	0.014	0.013	(-0.012, 0.039)	0.285
Sex	-0.548	0.179	(-0.916, -0.181)	0.005
PWS ³	-0.175	0.204	(-0.593, 0.244)	0.399

¹Partial regression coefficient: the change in BMR for a change in a specific variable adjusted for the other independent variables in the equation.

²Range of values that includes the population value of the coefficient.

³Grouping variable PWS was defined as follows: PWS patients = 1, control subjects = 0; the interaction variable $\text{PWS} \times \text{FFM}$ was not significant.

sleep than did sex- and bone age-matched control subjects. Previous reports indicating a lower than average BMR in this syndrome were based on studies of small groups of adult PWS patients and were without control groups (21, 26). In a more recent study that measured BMR in children and adolescents (13), 10 subjects with PWS were compared with a cohort of 60 healthy schoolchildren. Although the difference in BMR between groups was not significant, the difference decreased further after adjustment for age, sex, and FFM. Therefore, to study the relation between FFM and energy expenditure in PWS, a control group is needed that is matched for age and sex. However, most children with PWS have a delay in biological maturation, possibly because of the suggested hypothalamic insufficiencies. Calendar age often overestimates the physical development of a PWS child (8). Bone age measurement, as a means of assessing the rate of maturational change throughout the growing period, provides an estimate of physiologic maturation (27). Thus, in the present study, the control group was matched for sex and bone age.

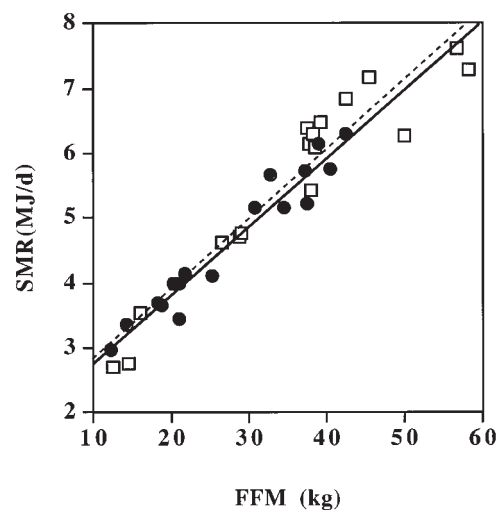


FIGURE 2. Sleeping metabolic rate (SMR) as a function of fat-free mass (FFM) for the Prader-Willi syndrome (PWS; ●) and control (□) groups. The regression equation for PWS patients: $\text{SMR} = 0.11 \text{ FFM} + 1.70$ ($R^2 = 0.94$); for control subjects: $\text{SMR} = 0.11 \text{ FFM} + 1.73$ ($R^2 = 0.91$).

TABLE 3

Results of multiple linear regression analysis of the influence of bone age, sex, weight, and Prader-Willi syndrome (PWS) on sleeping metabolic rate (SMR)

Variable	β Coefficient ¹	SE	95% CI β ²	P
Bone age	0.039	0.043	(-0.050, 0.128)	0.380
Sex	-0.505	0.149	(-0.810, 0.200)	0.002
Weight	0.052	0.006	(0.040, 0.064)	0.000
PWS ³	-0.380	0.157	(-0.701, 0.059)	0.022

¹Partial regression coefficient: the change in SMR for a change in a specific variable adjusted for the other independent variables in the equation.

²Range of values that includes the population value of the coefficient.

³Grouping variable PWS was defined as follows: PWS patients = 1, control subjects = 0; the interaction variable PWS \times weight was not significant.

To adjust BMR for FFM, BMR was plotted as a function of FFM. Because FFM explained >87% of the observed variability in BMR, the reduction in BMR must mainly be the result of a low FFM. Indeed, the similarity of the regression lines of the groups indicates that the relation between FFM and BMR in PWS was comparable with the relation in healthy children. In addition, there was a small, nonsignificant difference in intercepts between the 2 groups, but equal slopes for the lines. This finding differs from that of Hill et al (14), who found a lower BMR in PWS patients, even when adjusted for FFM, than in 2 groups of lean and obese control subjects. The authors suggested that persons with PWS have low energy expenditures early in life, but which become normal as these patients grow older and obesity becomes greater. In the present study, neither bone age nor FM significantly explained the observed variability in BMR with FFM in a multiple regression analysis. Moreover, PWS status was not important in predicting BMR when adjusted for FFM. A possible explanation for this conflicting result might be that this study used bioelectrical impedance analysis to meas-

ure FFM instead of a dilution technique. The high correlation between the skinfold-thickness measurements and the results of bioelectrical impedance analysis especially suggests that the latter may overestimate actual FFM because skinfold thicknesses are known to underestimate the amount of FM in PWS on account of the altered distribution of body fat (11). Another reason might be that the differences within and between studies are caused by the residual variability of the BMR measurement. Previous studies have reported that it was difficult for children with PWS to lay quietly for a BMR measurement, often resulting in no satisfactory measurement at all (13, 14). The measurement of SMR overnight in the respiratory chamber reduced the influence of agitation on the measurement. Thus, FFM explained 93% of the observed variability. Other variables such as FM, sex, and bone age, therefore, only played a minor role in the prediction of SMR. However, when body weight instead of FFM is used to predict SMR, the grouping variable PWS is significant, even when adjusted for bone age and sex, indicating that young PWS patients have a low SMR.

Thus, a low BMR and SMR must be the result of a low FFM. In the present study, FFM was significantly lower in the PWS group, both as an absolute value and when adjusted for height (FFMI). FFMI is an objective measure for comparing healthy subjects with patients with growth deficiency. Indeed, when corrected for height, the difference in FFM in the present study was even more significant ($P < 0.01$). Furthermore, it is also necessary to adjust FFM for the effect of FM because an increase in FM will induce an increase in FFM. When FFMI was plotted as a function of FMI, however, the slope of the PWS group was significantly lower than that of the control group, indicating that for each unit increase in FM the increase in FFM will be relatively lower in PWS patients.

The precise etiology of the reduction in growth of FFM in PWS is still unclear. The profound hypotonia in PWS might prevent the child from becoming physically active, resulting in low stimulation of muscle and bone tissue, consequently leading to a growth deficit of FFM.

A more general explanation relates the deficit in FFM to a dysfunction of the hypothalamic systems. Abnormalities of the somatotrophic axis would explain why most patients have a blunted growth hormone response to various provocative stimuli or show low 24-h growth hormone secretion (28–31). In addition, reduced concentrations of gonadotropins, consistent with hypogonadotropic hypogonadism, suggest a dysfunction of the hypothalamic-pituitary-gonadal axis (6). Although a structural hypothalamic lesion has not been found, a recent study found a complete absence of the posterior pituitary bright spot on magnetic resonance imaging, indicating disturbed functioning of the hypothalamic-hypophyseal system (7). The administration of growth hormone to subjects with PWS in several studies had a positive effect on height and reduced percentage body fat (29, 32, 33). Currently, prolonged trials of growth hormone treatment are underway to explore different dosage regimens and possible long-term adverse effects of these interventions.

In conclusion, this is the first report that shows energy expenditure to be lower at rest as well as during sleep in children and adolescents with PWS than in obese control subjects matched for bone age and sex. The reduced energy expenditure is the result of low FFM, both as an absolute value and when adjusted for height. There was no significant difference in energy expenditure between normal children and those with PWS when BMR or SMR was adjusted for FFM.

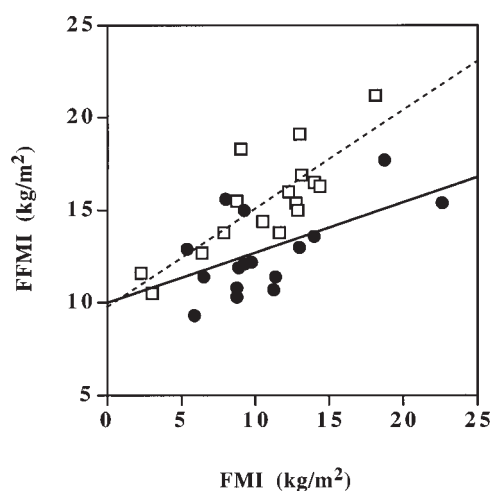


FIGURE 3. Fat-free mass index (FFMI) as a function of fat mass index (FMI) plotted for the Prader-Willi syndrome (PWS; ●) and control (□) groups. The regression line of the PWS patients is significantly different from the regression line of the control subjects, $P < 0.001$ (multiple linear regression analysis).

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